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# MPO (Urine / Stool) ELISA

Catalog Number:

MPO39-K01 (1 x 96 wells)

*For Research Use Only. Not for use in diagnostic procedures.*

*v. 6.0 (27 DEC 23)*

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## **INTENDED USE**

The Eagle Biosciences Myeloperoxidase (MPO) ELISA Assay kit is intended for use in the quantitative determination of human myeloperoxidase (MPO) levels in stool and urine samples. The test is useful for detecting elevated levels of myeloperoxidase in stool samples, which may serve as a sensitive predictor for inflammatory activities in the gastrointestinal tract. The Eagle Biosciences MPO ELISA Assay Kit is for research use only and not to be used for diagnostic procedures.

## **SUMMARY OF PHYSIOLOGY**

Myeloperoxidase (MPO) is a specific polymorphonuclear enzyme that is most abundantly expressed in neutrophil granulocytes. It functions in the oxygen-dependent killing of microorganisms, and is released from primary granules of neutrophils during acute inflammation. MPO is the product of a single gene, which is about 11 kb in size, composed of 11 introns and 12 exons, and located in the long arm of chromosome 17 in segment q12-24. The mature 150 kDa MPO protein is a dimer consisting of two 15 kDa light chains and two heavy chains of variable degrees of glycosylation.

MPO activity was found to be linearly related to the number of neutrophil cells. Since neutrophils play a predominant role in inflammatory and immune reactions in inflammatory bowel disease (IBD), and MPO has been observed both in the intestinal mucosa and the gut lavage, the determination of MPO in stool sample provides one of the most sensitive and promising biomarkers in predicting disease severity. This assay utilizes a specific monoclonal antibody to capture MPO in test sample to ensure that only myeloperoxidase is detected.

## **ASSAY PRINCIPLE**

This Myeloperoxidase (MPO) ELISA Assay kit is designed, developed and produced for the quantitative measurement of human myeloperoxidase in stool samples. The assay utilizes the two-site "sandwich" technique with selected antibodies that bind to different epitopes of myeloperoxidase.

Assay standards, controls and extracted patient samples are added directly to wells of a microtiter plate that is coated with antibody to myeloperoxidase. After an incubation period, the plate is washed and horseradish peroxidase (HRP)-conjugated human myeloperoxidase antibody is added to each well. After the second incubation period, a "sandwich" of solid-phase monoclonal antibody – human myeloperoxidase – HRP-conjugated antibody" is formed. The unbound antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction and the absorbances are then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human myeloperoxidase in the test sample. A standard curve is generated by plotting the absorbance versus the respective human myeloperoxidase concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of human myeloperoxidase in test samples is determined directly from this standard curve.



## **REAGENTS: Preparation and Storage**

This Myeloperoxidase (MPO) ELISA Assay test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

**Prior to use allow all reagents to come to room temperature.** Reagents from different kit lot numbers should not be combined or interchanged.

### **1. Myeloperoxidase Antibody Coated Microplate**

One microplate with twelve by eight strips (96 wells total) coated with myeloperoxidase antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

### **2. Myeloperoxidase Tracer Antibody**

One vial containing 0.6 mL HRP-labeled anti-human myeloperoxidase antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent (Cat. 30605) before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

### **3. ELISA Wash Concentrate**

One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with **870 mL** of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

### **4. ELISA HRP Substrate**

One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

### **5. ELISA Stop Solution**

One bottle contains 12 mL of 2N Hydrochloric Acid (HCl). This reagent may be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

### **6. Myeloperoxidase Standard Concentrate**

One vial containing human myeloperoxidase in a lyophilized bovine serum-based matrix with a non-azide preservative. **Refer to the vial for exact concentration of the standard.** This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

### **7. Myeloperoxidase Controls**

Three vials containing human myeloperoxidase in a lyophilized bovine serum-based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box.



### **8. Tracer Antibody Diluent**

One vial containing 12 mL ready-to-use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

### **9. Extraction Buffer**

One bottle containing 30 mL of 20-fold concentrate. Before use, the contents must be diluted with 570 mL of demineralized water and mixed well. Upon dilution, this yields a ready-to-use Extraction Buffer for fecal sample extraction and dilution. The diluted Extraction Buffer may be stored at room temperature and is stable until the expiration date on the kit box.

### **SAFETY PRECAUTIONS**

The reagents of the Myeloperoxidase (MPO) ELISA Assay kit must be used for research use only. Source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or hydrochloric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Hydrochloric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices. .

### **MATERIALS REQUIRED BUT NOT PROVIDED**

1. Fecal sample collection tube.
2. Precision single channel pipettes capable of delivering 50 µL, 100 µL, 500 µL, etc.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable plastic 100 mL and 1000 mL bottle with caps.
5. Aluminum foil.
6. Deionized or distilled water.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 405/650 nm.

### **SPECIMEN COLLECTION**

#### **1. Fecal sample collection**

(1) Only one fecal sample is required. Fresh fecal sample must be collected by using Eagle Biosciences Fecal MPO Sample Collection Tube (Cat. No. MPO35-C50). This tube is specially designed for easy collection of a substantially small amount of fecal sample into the tube pre-filled with sample extraction buffer. The collected fecal sample may be transported at ambient temperature, stored at 2-8°C and tested within 3 days. Fecal sample may be stored below -20°C for a longer storage period. Avoid more than three freeze - thaw cycles for each specimen.

**The validation data of this test were generated by using Eagle Biosciences Fecal MPO Sample Collection Tube! To order this tube, please order Fecal MPO Sample Collection kit (Cat. No. MPO35-C50) and each kit contains 50 tubes that are pre-filled with fecal sample extraction buffer. A different myeloperoxidase test result may be obtained by using a different type of fecal sample collection tube and extraction buffer.**



(2) It is an alternative to collect fecal sample with a commercial stool sample collection device. The collected sample can be stored at 2-8°C for up to 6 days. The collected sample should be diluted in two steps with 1:40 and 1:9 before measurement. Following is a detailed sample extraction process:

- (a) Label and tare an empty polypropylene tube together with an inoculation loop.
- (b) Weigh 50 – 100 mg of stool using the inoculation loop by placing it into the pre-tared tube.
- (c) Record the net amount of sample and break the inoculation loop; leave the lower part of the loop in the tube.
- (d) For every 1 volume of the stool, add 39 volume of Extraction buffer into the tube (stool volume calculation: 100 mg stool = 100 µl stool). The following is an easy to use reference extraction procedure.

| <b>Fecal Sample Weight (mg)</b> | <b>Extraction Buffer Volume (mL)</b> |
|---------------------------------|--------------------------------------|
| 50 - 54                         | 2.0                                  |
| 55 - 59                         | 2.2                                  |
| 60 - 64                         | 2.4                                  |
| 65 - 69                         | 2.6                                  |
| 70 - 74                         | 2.8                                  |
| 75 - 79                         | 3.0                                  |
| 80 - 84                         | 3.2                                  |
| 85 - 89                         | 3.4                                  |
| 90 - 94                         | 3.6                                  |
| 95 - 99                         | 3.8                                  |
| 100 - 104                       | 4.0                                  |

(e) Vortex to dissolve stool sample. Let the sample set at room temperature vertically for 30 min for sedimentation or centrifuge the sample at 3000 x g for 5 minutes.

(f) Transfer 0.15 ml clear supernatant (no particles) to a clean tube with 1.2 ml Extraction Buffer. Mix the sample by gently vortexing. This extracted sample is ready to be measured for fecal myeloperoxidase.

## **2. Urine sample collection**

A standard urine collection container must be used to collect a fresh and random urine sample.

## **ASSAY PROCEDURE**

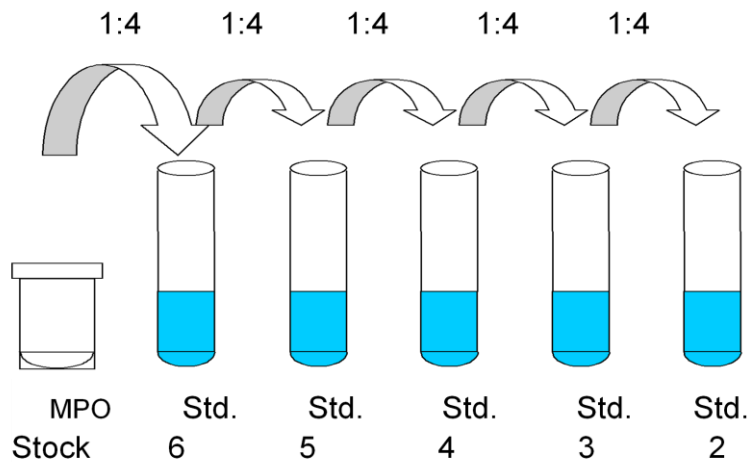
### **1. Reagent Preparation**

- (1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
- (2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.
- (3) Reconstitute assay standard by adding 2.0 mL of demineralized water to standard vial. Separately, reconstitute controls by adding 1.0 mL of demineralized water to control vials. Allow the standard and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solid is dissolved completely prior to use. These reconstituted standard and controls may be stored at 2-8°C for up



o 3 days or at  $-10^{\circ}\text{C}$  or below for long-term storage. Do not exceed 3 freeze-thaw cycles.

(4) Dilute the reconstituted standard concentrate 1:4 using the fecal sample extraction buffer to obtain a level six standard by mixing the concentrated MPO standard (Cat. 30601) with the fecal sample extraction buffer. For example: mix 300  $\mu\text{L}$  of concentrated MPO standard with 900  $\mu\text{L}$  of the extraction buffer. Continue diluting standards down to level two as it is shown below. Level one standard is the fecal sample extraction buffer.



(5) Test Configuration

| ROW | STRIP 1 | STRIP 2 | STRIP 3  | STRIP 4  |
|-----|---------|---------|----------|----------|
| A   | STD 1   | STD 5   | C 3      | SAMPLE 4 |
| B   | STD 1   | STD 5   | C 3      | SAMPLE 4 |
| C   | STD 2   | STD 6   | SAMPLE 1 | SAMPLE 5 |
| D   | STD 2   | STD 6   | SAMPLE 1 | SAMPLE 5 |
| E   | STD 3   | C 1     | SAMPLE 2 | SAMPLE 6 |
| F   | STD 3   | C 1     | SAMPLE 2 | SAMPLE 7 |
| G   | STD 4   | C 2     | SAMPLE 3 | SAMPLE 7 |
| H   | STD 4   | C 2     | SAMPLE 3 | Etc.     |



- (6) Place a sufficient number of myeloperoxidase antibody coated microwell strips in a holder to run human myeloperoxidase standards, controls and unknown samples in duplicate.
- (7) Prepare Tracer Antibody working solution by 1:21 fold dilution of the Myeloperoxidase Tracer Antibody by adding the tracer antibody into the Tracer Antibody Diluent. Following is a table that outlines the relationship of strips used and antibody mixture prepared.

| <b>Dilution Scheme</b> | <b>Tracer Antibody Diluent</b> | <b>Tracer Antibody</b> |
|------------------------|--------------------------------|------------------------|
| <b>1</b>               | 1 mL                           | 50 µL                  |
| <b>2</b>               | 2 mL                           | 100 µL                 |
| <b>3</b>               | 3 mL                           | 150 µL                 |
| <b>4</b>               | 4 mL                           | 200 µL                 |
| <b>5</b>               | 5 mL                           | 250 µL                 |
| <b>6</b>               | 6 mL                           | 300 µL                 |
| <b>7</b>               | 7 mL                           | 350 µL                 |
| <b>8</b>               | 8 mL                           | 400 µL                 |
| <b>9</b>               | 9 mL                           | 450 µL                 |
| <b>10</b>              | 10 mL                          | 500 µL                 |
| <b>11</b>              | 11 mL                          | 550 µL                 |
| <b>12</b>              | 12 mL                          | 600 µL                 |

*Note: this antibody working solution should be freshly prepared just before pipetting the tracer antibody to the washed wells.*

## **2. Patient Sample Preparation**

- (a) If the Eagle Biosciences Fecal Sample Collection Tube is used, there is no sample preparation required. In case that the collection tube is not used, please refer to the specimen collection section.
- (b) Each urine sample must be diluted 1:5 using the fecal sample extraction buffer.

## **3. Assay Procedure:**

- (1) Add 100 µL of Standards, Controls and extracted patient samples into the designated microwells.
- (2) Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for 1.5 hr. ± 5 minutes at 400 to 450 rpm.
- (3) Just prior to the end of the incubation time, dilute the proper amount of Tracer Antibody for the assay.
- (4) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (5) Add 100 µL of above Tracer Antibody to each well.
- (6) Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for 45 minutes ± 5 minutes at 400 to 450 rpm.
- (7) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.



- (8) Add **100 µL** of ELISA HRP Substrate into each of the wells.
- (9) Cover the plate with aluminum foil to or other material to avoid exposure to light. Incubate plate static, at room temperature for **20 minutes**.
- (10) Immediately add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.
- (11) Read the absorbance at 405 nm with reference filter at 620 nm or 650 nm.

### PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. An orbital mixer with a larger orbit radius (e.g. > 1 cm) may be used at speeds of 150 to 200 rpm.
7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
9. If adapting this assay to automated ELISA system such as DS-2, a procedural validation is necessary if there is any modification of the assay procedure.

### INTERPRETATION OF RESULTS

It is recommended to use a point-to-point or 4-parameter standard curve fitting.

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 standard (0 µg/g) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The fecal human myeloperoxidase concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.

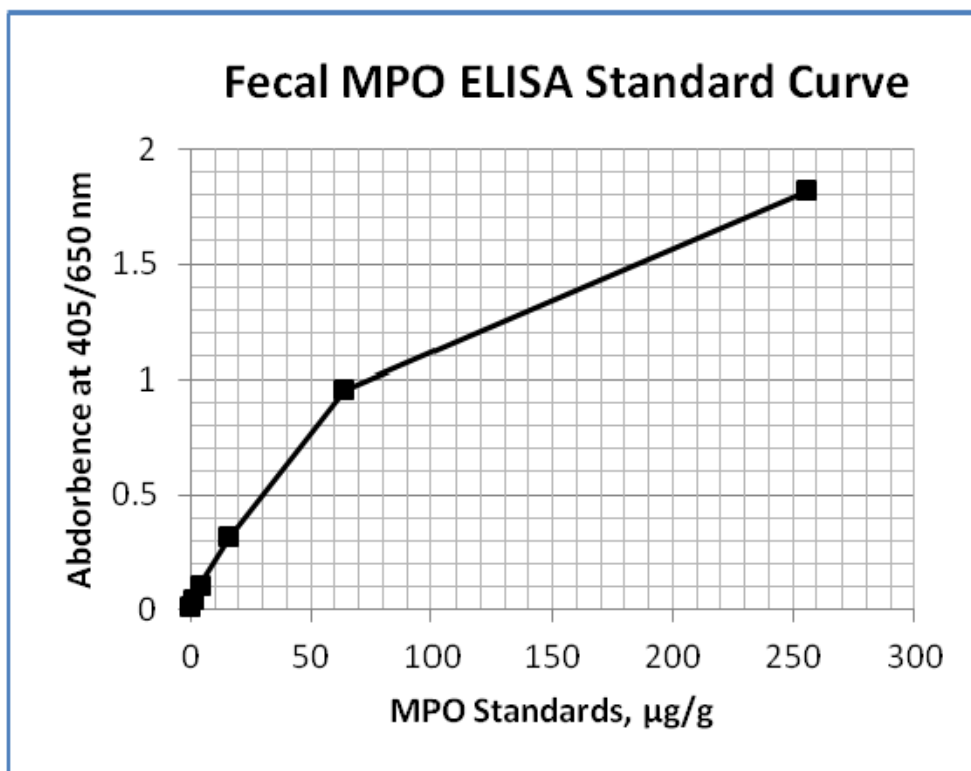
### EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this fecal human myeloperoxidase ELISA are represented. This curve should not be used in lieu of standard curve run with each assay.





| Well I.D.       | OD 405/650 nm Absorbance |         |           | Results    |
|-----------------|--------------------------|---------|-----------|------------|
|                 | Readings                 | Average | Corrected |            |
| Std-1: 0 µg/g   | 0.015<br>0.016           | 0.015   | 0.000     |            |
| Std-2: 1 µg/g   | 0.041<br>0.042           | 0.042   | 0.027     |            |
| Std-3: 4 µg/g   | 0.105<br>0.105           | 0.105   | 0.090     |            |
| Std-4: 16 µg/g  | 0.305<br>0.325           | 0.315   | 0.300     |            |
| Std-5: 64 µg/g  | 0.960<br>0.950           | 0.955   | 0.940     |            |
| Std-6: 256 µg/g | 1.810<br>1.826           | 1.818   | 1.803     |            |
| Control 1       | 0.080<br>0.083           | 0.082   | 0.067     | 2.9 µg/g   |
| Control 2       | 0.257<br>0.267           | 0.262   | 0.247     | 12.95 µg/g |
| Control 3       | 0.706<br>0.732           | 0.719   | 0.704     | 46.3 µg/g  |





## EXPECTED VALUES

Stool and urine samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA using our stool sample collection tube filled with extraction buffer.

The recommended normal cut-off for fecal myeloperoxidase concentration by using this ELISA and sample collection system is  $<2 \mu\text{g/g}$ . We strongly recommend for each clinical laboratory to establish its own normal cut-off level by measuring normal stool samples with this ELISA and sample collection system.

Please be aware that patients with recent diarrhea would give a much higher level of fecal Myeloperoxidase. Consuming spicy food or alcohol may also cause intestinal irritation resulting in an abnormal fecal Myeloperoxidase level.

MPO concentration for urine sample is read from the assay standard curve and must be multiplied by 5 (dilution factor) to get the true MPO concentration in urine. The recommended normal cut-off for urine myeloperoxidase concentration by using this ELISA is  $<20 \text{ ng/mL}$ .

**Note:**            **MPO ng/mL X 0.36 = MPO  $\mu\text{g/g}$**

**MPO  $\mu\text{g/g}$  X 2.78 = MPO ng/mL**

*Please program ELISA reader by selecting assay standards concentration either in “ $\mu\text{g/g}$ ” or “ng/mL to avoid manual calculation!*

## LIMITATION OF THE PROCEDURE

1. A strong positive of fecal myeloperoxidase is likely to indicate a more significant clinical pathological condition of a patient. However, a low positive of fecal myeloperoxidase does not indicate a lesser possibility of inflammation.
2. A normal fecal myeloperoxidase level does not rule out the presence of any gastrointestinal diseases such as IBD.
3. For sample values reading greater than the highest standard, it is recommended to re-assay samples with dilution (i.e. 1:10 or 1:100 with Extraction Buffer).
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

## QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known positive levels of MPO. We recommend that all assays include the lab's own control samples in addition to those provided with this kit.

## PERFORMANCE CHARACTERISTICS

### Sensitivity

The analytical sensitivity (LLOD) of the human myeloperoxidase ELISA as determined by the 95% confidence limit on 16 duplicate determination of zero standard is approximately  $0.15 \mu\text{g/g}$ .

### High Dose “hook” effect

This assay has showed that it did not have any high dose “hook” for myeloperoxidase level up to  $25,000 \mu\text{g/g}$  in extraction buffer.

### Precision

The intra-assay precision was validated by measuring three sample extracts in a single assay with 12 replicate determinations.



| Mean Myeloperoxidase Value ( $\mu\text{g/g}$ ) | CV (%) |
|--|--------|
| 14.0   | 5.2    |
| 32.0   | 1.7    |
| 121.5  | 5.2    |

The inter-assay precision was validated by measuring three samples in duplicate in 8 individual assays.

| Mean Myeloperoxidase Value ( $\mu\text{g/g}$ ) | CV (%) |
|--|--------|
| 8.95   | 5.0    |
| 29.9   | 6.3    |
| 101.4  | 10.3   |

#### Linearity

Three fecal samples were collected by using fecal collection tubes (Cat. No. 30356), spiked with various amounts of myeloperoxidase, diluted with assay buffer and tested. The results of myeloperoxidase percent recovery value in  $\mu\text{g/g}$  are as follows:

| DILUTION      | OBSERVED VALUE ( $\mu\text{g/g}$ ) | RECOVERY % |
|---------------|------------------------------------|------------|
| <b>Neat A</b> | 10.2                               | -          |
| 1:2           | 5.5                                | 107        |
| 1:4           | 3.2                                | 123        |
| 1:8           | 1.8                                | 135        |
| <b>Neat B</b> | 34.6                               | -          |
| 1:2           | 16.7                               | 97         |
| 1:4           | 10.0                               | 115        |
| 1:8           | 5.8                                | 132        |
| <b>Neat C</b> | 108.7                              | -          |
| 1:2           | 56.3                               | 103        |
| 1:4           | 32.9                               | 121        |
| 1:8           | 14.3                               | 105        |



### Spike Recovery

Three fecal extracts and three assay standards (4, 16, 64 µg/g) were combined at equal volumes and tested. The results are as follows:

| DILUTION      | OBSERVED VALUE (µg/g) | EXPECTED VALUE (µg/g) | RECOVERY % |
|---------------|-----------------------|-----------------------|------------|
| <b>Neat A</b> | 7.4                   | -                     | -          |
| Std-3         | 6.1                   | 5.7                   | 107        |
| Std-4         | 11.0                  | 11.7                  | 94         |
| Std-5         | 31.0                  | 35.7                  | 87         |
| <b>Neat B</b> | 15.0                  | -                     | -          |
| Std-3         | 11.5                  | 9.5                   | 121        |
| Std-4         | 15.5                  | 15.5                  | 100        |
| Std-5         | 38.6                  | 39.5                  | 98         |
| <b>Neat C</b> | 37.3                  | -                     | -          |
| Std-3         | 22.7                  | 20.7                  | 110        |
| Std-4         | 31.8                  | 26.7                  | 119        |
| Std-5         | 49.3                  | 50.7                  | 97         |

### REFERENCES

1. Saiki T. Myeloperoxidase concentrations in the stool as a new parameter of inflammatory bowel disease. Kurume Med a. 1998;45:69-73.
2. Angriman I, et al. Enzymes in feces: useful markers of chronic inflammatory bowel disease. Clin Chim Acta. 2007;381(1):63-8.

## Myeloperoxidase ELISA: Condensed Assay Protocol

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1. 100  $\mu$ L Calibrators, controls and extracted patient samples

*Incubate @ RT for 90 min  
on ELISA plate  
shaker Wash 5 x*



2. 100  $\mu$ L Tracer Antibody

*Incubate @ RT for 45  
min on ELISA plate  
shaker Wash 5 x*



3. 100  $\mu$ L TMB Substrate

*Incubate @ RT for 20  
min static*



4. 100  $\mu$ L Stop Solution

*Immediately*



5. Read absorbance at 405/650 nm

### Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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*For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.*